

Developmental Changes in the Hormonal Regulation of Rat Testis Sertoli Cell Adenylyl Cyclase*

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ABSTRACT. The stimulatory effects of FSH on Sertoli cell functions such as cAMP accumulation, protein kinase activation, and RNA and protein synthesis wane during testis maturation. However, FSH receptors increase with age and addition of cAMP stimulates these biochemical events in Sertoli cells from animals of any age. In order to determine if this loss of responsiveness to FSH was due to an inability to stimulate adenylyl cyclase, the hormonal responsiveness of this enzyme was investigated as a function of testicular development. In agreement with intact cell studies, adenylyl cyclase activity was found to be stimulated by FSH 2- to 3-fold in homogenates of testes from immature (5–20 days of age) Sertoli cell-enriched rats, while no stimulation of the enzyme by FSH was observed in similar homogenates from Sertoli cell-enriched animals 20 days of age or older. The possibility of a decrease in enzyme sensitivity to the gonadotropin as

a function of maturation was ruled out by dose-response studies. Catalytic activity of the enzyme was retained with increasing animal age as evidenced by the ability of fluoride (10 mM) to stimulate basal activity 4-fold. Hormonal responsiveness of the Sertoli cell adenylyl cyclase of mature animals could be restored, however, either by addition of the nonmetabolizable guanosine 5'-triphosphate analog, 5'-guanylyl-imidodiphosphate to homogenates or by preparation of membrane particles. We found that 5'-guanylyl-imidodiphosphate selectively potentiated FSH effects on cyclase in testicular homogenates from mature animals while having no effect on the relative degree of hormone stimulation in homogenates from immature animals, and that in contrast to homogenates, testicular membrane preparations retain their FSH responsiveness upon animal maturation. (*Endocrinology* 109: 1270, 1981)

A TEMPORAL relationship has been established between binding of FSH to isolated membranes prepared from Sertoli cells (1–3), activation of adenylyl cyclase (4–6), and inhibition of cyclic nucleotide phosphodiesterase (6, 7). These altered enzyme activities have been shown to result in an elevation in the intracellular levels of cAMP in whole cell preparations from testes of immature rats (8–11), hypophysectomized animals (12), cryptorchid animals (11), x-irradiated animals (6, 7, 13), and isolated Sertoli cells in tissue culture (14, 15). These biochemical responses to FSH in the Sertoli cell have been shown to be age dependent. Maximum sensitivity to this gonadotropin occurs between 12 and 15 days of age, and by 20 days of age little effect can be seen.

Lack of FSH sensitivity in the Sertoli cell cannot be attributed to decreased receptors since receptor numbers have been shown to increase as a function of testis maturation (16, 17). Secondly, all of the biochemical events elicited by FSH in the Sertoli cell of immature

rats, with the possible exception of transcriptional events, can be mimicked in Sertoli cells from mature animals by cAMP derivatives or cholera toxin, which nonspecifically increases intracellular cAMP levels (18, 19). Finally, recent studies by Steinberger *et al.* (20) demonstrate that in the presence of FSH and 1-methyl-3-isobutylxanthine, a phosphodiesterase inhibitor, cAMP accumulation in Sertoli cells from mature rats is only 10% of the maximal levels elicited by FSH alone in immature Sertoli cells, suggesting an aberration in adenylyl cyclase function.

We have investigated the sensitivity of adenylyl cyclase to FSH and fluoride, a nonspecific activator of adenylyl cyclases (for review, see Ref. 21), in testicular homogenates and membrane preparations of Sertoli cell-enriched (SCE) testes as a function of testes development. The response of the adenylyl cyclase system to FSH was found to decrease with increasing animal age when determined in total homogenates but not when assayed in membrane particles. Fluoride sensitivity of the testis enzyme was maintained regardless of age.

Since a guanosine 5'-triphosphate (GTP) binding protein has been reported to be involved in hormone receptor-cyclase coupling of many systems (22–27), the effect of the guanyl nucleotide analog, guanylyl 5'-imidodiphosphate [GMP-P(NH)P] on hormonal sensitivity of aden-

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adenylyl cyclase was determined in homogenate and membrane preparations from immature and mature SCE testes. Potentiation of adenylyl cyclase response to FSH in the presence of GMP-P(NH)P was observed in testicular homogenates from mature animals.

Materials and Methods

Materials

The ovine FSH preparation NIH-S10 or S12 (LH contamination, 0.01 U/mg by bioassay) and the ovine LH preparation NIH-S8 (FSH contamination, 0.05 U/mg by bioassay) were gifts from the NIAMDD Pituitary Hormone Distribution Program (Bethesda, MD). Highly purified FSH was kindly provided by Dr. K. Cheng (University of Manitoba, Canada). The peptide hormones were dissolved in 0.1% bovine serum albumin and frozen in aliquots. Testosterone propionate was obtained from Elkins-Sinn, Inc. (Cherry Hill, NH). Bis-Tris-propane, free base (1,3-bis[Tris-(hydroxymethyl-methylamino)] propane, free base); ATP; either Tris or sodium salt; cAMP, myokinase (crystalline in saturated ammonium sulfate); guanylylimidodiphosphate tetralithium salt [GMP-P(NH)P]; and EDTA were purchased from Sigma Chemical Company (St. Louis, MO). Phosphocreatine and creatine phosphokinase (rabbit muscle) were purchased from Calbiochem (La Jolla, CA). 1-Methyl-3-isobutylxanthine was purchased from Aldrich (Milwaukee, WI). All other reagents were of analytical grade. Dowex 10-X4 (AG, 200–400 mesh, H⁺ form) was purchased from Bio-Rad (Richmond, CA). [³H]cAMP was obtained from Schwartz-Mann (Orangeburg, NY) and the [α -³²P]ATP was synthesized by the method of Walseth and Johnson (28).

Animals

SCE testes were prepared as described previously. Pregnant rats obtained from the Holtzman Company (Madison, WI) were irradiated at 19 days of gestation with 125 rads (whole body) from a ⁶⁰Co source. The gonocytes in the male fetuses show an increased radiosensitivity and degenerate during the definitive postnatal division, resulting in a sterile testis. The testes were devoid of germ cells but contained predominantly Sertoli cells as well as a normal complement of myoid and Leydig cells (6, 7, 13). At all ages the Sertoli cells constituted greater than 93% of the total testis mass. Maximal testis weight is achieved at 40 days of age in these animals (6). At all ages the increased weight is predominantly due to an increase in the number and/or size of the Sertoli cells (6). After weaning at 21 days of age, the rats were given water and Purina Lab Chow *ad libitum*. Rats were killed by cervical dislocation at the age specified in the text and their testes were removed.

Tissue preparation

Testes were homogenized in 20 volumes (wt/vol) of 27% (wt/wt) sucrose, 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA. Homogenization was accomplished in a 7-ml Dounce homogenizer (Kontes Co., Vineland, NY) using 10 up/down strokes with an "A" pestle and 20 up/down strokes with a "B" pestle. This

preparation was the source of adenylyl cyclase in homogenate studies. When membrane preparations were desired, the homogenate was centrifuged 5 min at 600 × *g*_{av}. The nuclear pellet was discarded and the supernatant suspension was transferred to a second centrifuge tube and recentrifuged 30 min at 12,000 × *g*_{av} in a JA-20 rotor (Beckman Instruments, Palo Alto, CA). The resulting pellet was resuspended in 20 volumes (with respect to original tissue weight) and subjected to a second centrifugation at 12,000 × *g*_{av} for 30 min in a JA-20 rotor (Beckman). The supernatant was discarded and the pellet ("washed membrane particles") was resuspended in 5 volumes (with respect to original tissue weight). Aliquots of homogenate and membrane preparations were assayed for protein content and enzymatic activities as described in sections below.

Assay of adenylyl cyclase

For both homogenates and membrane particles, the conditions of assay were optimized to obtain a maximal response to FSH. Thus, unless indicated otherwise, aliquots (20 μ l) of homogenate or membrane particle preparation (30–50 μ g protein) were assayed in triplicate for adenylyl cyclase activity in a final volume of 50 μ l containing 0.4 mM ATP (homogenates) or 1.0 mM ATP (membranes) with 5 × 10⁶ cpm [α -³²P]ATP, 5.0 mM MgCl₂, 1.0 mM EDTA, 1.0 mM [³H]cAMP (approximately 10,000 cpm), 20 mM creatine phosphate, 0.2 mg/ml creatine kinase, 0.1 mg/ml myokinase, and 25 mM bis-Tris-propane HCl buffer, pH 8.5. Incubations were performed at 37°C for 10 min. The reaction was stopped with 0.1 ml 10 mM cAMP, 40 mM ATP, followed immediately by boiling for 3.5 min. The [³²P]cAMP formed and [³H]cAMP present as recovery marker were isolated by the method of Salomon *et al.* (29) and quantified by liquid scintillation counting. Recovery of cAMP was 50–60% as determined from [³H]cAMP radioactivity. Results were corrected to 100% recovery and expressed as picomoles cAMP formed per min/mg protein. Under the assay conditions described above, levels of added ATP were maintained by better than 85% in incubations lasting up to 30 min, and levels of added 10 μ M [α -³²P]GTP were maintained by better than 85% in incubations lasting up to 15 min provided ATP concentrations were at least 0.4 mM in studies with homogenates and 1.0 mM in studies with membrane particles. Levels of nucleotides remaining were determined as described earlier (30). It should be noted that these assay conditions are markedly different than those required for LH stimulation of Leydig cell adenylyl cyclase as described by Dufau *et al.* (31).

Assay of protein

The protein content of various samples was determined by the method of Lowry *et al.* (32) using bovine serum albumin as standard.

Results

Effect of development on responsiveness of testicular adenylyl cyclase to FSH

The responsiveness of adenylyl cyclase to FSH was determined in SCE testis homogenates from animals of

different ages (Fig. 1). Maximal stimulation of adenylyl cyclase by FSH was evident at 9–11 days of age. Activity was increased from 16 pmol cAMP formed/min·mg protein to 50 pmol cAMP formed/min·mg protein in the presence of 50 μ g/ml NIH-FSH-S12 ($P < 0.001$). With increasing animal age, basal activity declined, so that by 45 days of age basal activity was 30% of that seen in the immature animal. The ability of FSH to stimulate cyclase also decreased as a function of animal age and did so more than basal activity. As shown under the assay conditions used here, no significant stimulation of cyclase activity by FSH was observed in testicular homogenates from SCE animals 24 days of age and older. This loss of responsiveness to FSH of adenylyl cyclase in homogenates was independent of the concentration of ATP used as substrate in the assay. In separate experiments it was determined that adenylyl cyclase activity in homogenates of SCE testes from 40-day-old rats assayed at 1.0 mM ATP was 6 ± 1 and 7 ± 2 pmol/min·ml protein (mean \pm SE of 3 experiments) in the absence and presence of FSH, respectively.

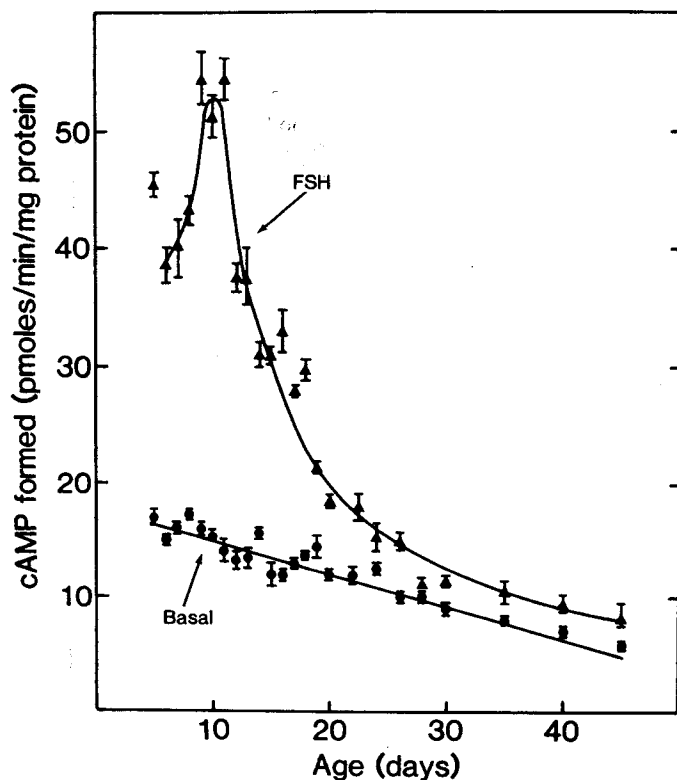


FIG. 1. FSH responsiveness of SCE testis homogenate adenylyl cyclase as a function of animal age. Adenylyl cyclase activity in testicular homogenates of SCE animals aged from 5–45 days old was determined in the absence (●) and presence (▲) of 50 μ g/ml NIH-FSH-S12. Assay conditions were as described in *Materials and Methods*. Means \pm SE from three separate experiments are represented. The best fit line for the basal data was determined by linear regression analysis. The correlation coefficient was 0.88.

Effect of purified FSH, LH, and testosterone on adenylyl cyclase activity

Purified bovine FSH (supplied by Dr. K. Cheng with a reported potency of 120 NIH-FSH-S12 units by bioassay), NIH-LH-S8, and testosterone propionate were tested for their ability to stimulate adenylyl cyclase in testicular homogenates from 12-day-old SCE animals. Results from a representative experiment are shown in Fig. 2. Purified FSH stimulated adenylyl cyclase activity in a dose-dependent manner (*panel A*). Half-maximal activation of the enzyme occurred at 250 ng/ml FSH. Maximal activation of activity was achieved with 1 μ g/ml FSH and consisted of a change from 8.0 pmol cAMP to 28 pmol cAMP/min·mg protein. A FSH preparation from NIH (S-12) stimulated activity 2.4-fold at 50 μ g/ml with half-maximal activation being obtained at approximately 3 μ g/ml (data not shown). NIH-LH-S8 had no demonstrable effect on adenylyl cyclase activity (*panel*

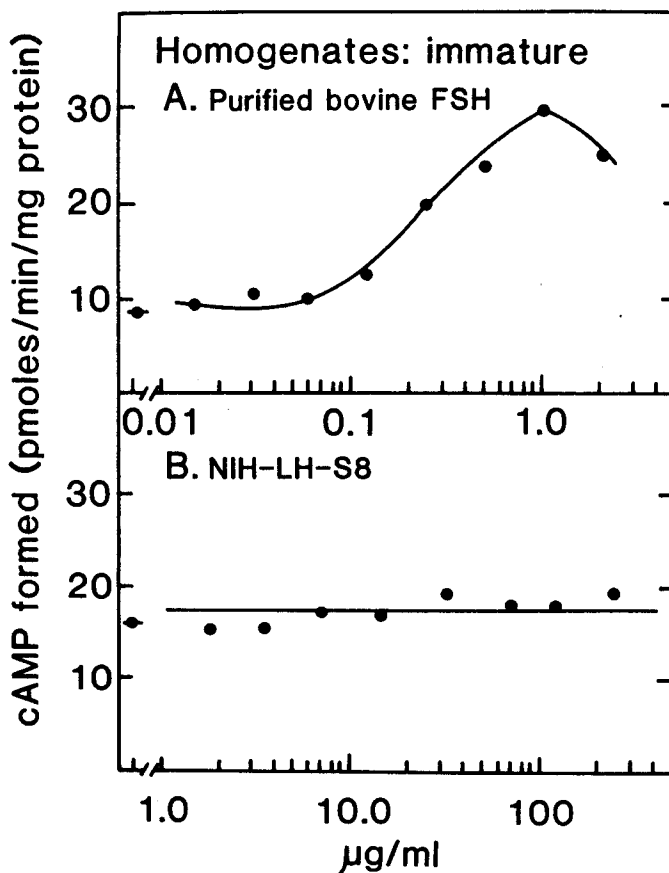


FIG. 2. Effect of purified FSH and of LH on SCE testis adenylyl cyclase. Adenylyl cyclase activity in testicular homogenates prepared from 12-day-old SCE animals was determined in the presence of varying concentrations of purified bovine FSH (reported potency 120X NIH-FSH-S12) or NIH-LH-S8. For other details see *Materials and Methods*. (In this and the following figures the data are the mean of triplicate determinations; representative experiments are shown, but each was performed at least 3 times.)

B) over a concentration range of 0.45–250 $\mu\text{g/ml}$. Similarly, in this and other experiments testosterone propionate had no effect on activity when tested over a concentration range of 0.125–150 $\mu\text{g/ml}$ (not shown).

Effect of FSH in testicular homogenates from mature SCE animals

The ability of varying concentrations of NIH-FSH-S12 ($1\text{--}4 \times 10^3 \mu\text{g/ml}$) to activate adenylyl cyclase in testicular homogenates from 43-day-old SCE animals was tested. Results from one such experiment are shown in Fig. 3. Cyclase activity was not stimulated by FSH at any concentration tested. Lack of cyclase responsiveness to FSH in these preparations can not be attributed to a change in dose dependency of the hormonal effect, for if it were, a hormonal effect should have become apparent at very high concentrations of added FSH.

Time course of adenylyl cyclase activity in testicular homogenates of immature and mature SCE animals

The rate of cAMP production by adenylyl cyclase was determined in testicular homogenates from 12- and 90-day-old SCE animals to assure that an age-dependent alteration in enzyme lability was not artifactually masking FSH stimulation of the enzyme (Fig. 4). Cyclase activity in preparations from immature rats was linear for 15 min in the absence or presence of 50 $\mu\text{g/ml}$ NIH-FSH-S12 (*panel A*). Activity was stimulated throughout the time course, 3.7-fold at 10 min. In contrast, cyclase activity in preparations from mature rats was linear for 15 min but was not significantly stimulated in the presence of FSH at any of the times tested (*panel B*).

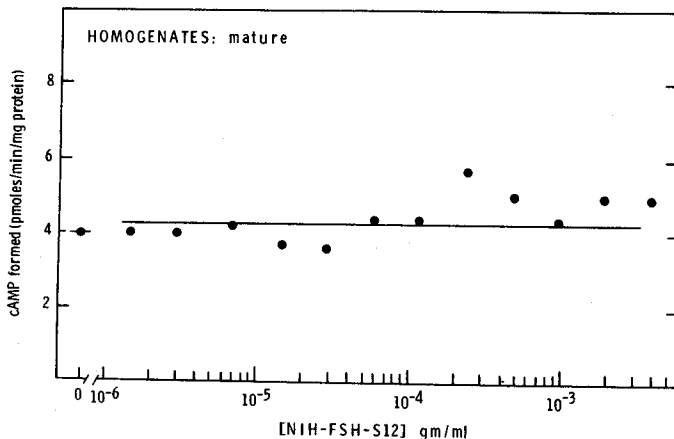


FIG. 3. Effect of NIH-FSH-S12 on adenylyl cyclase activity in testis from mature SCE animals. Adenylyl cyclase activity in testicular homogenates from 43-day-old SCE rats was determined in the presence of varying concentrations of NIH-FSH-S12. Assay conditions were as described in *Materials and Methods*.

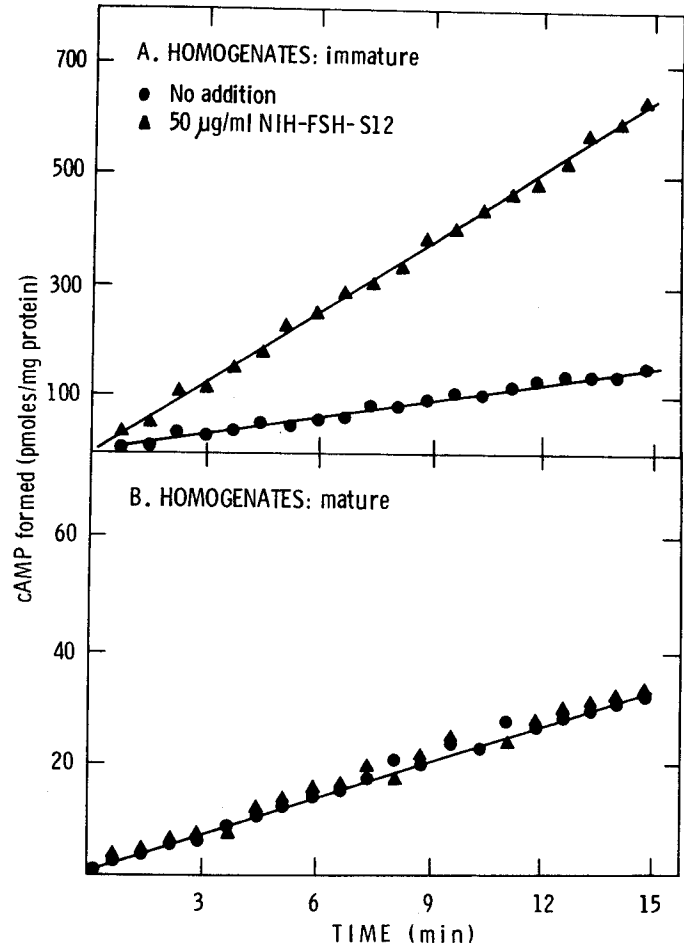


FIG. 4. Time courses of basal and hormone-stimulated adenylyl cyclase activities were determined in testicular homogenates prepared from 12-day-old (A) and 90-day-old (B) SCE rats. Homogenates were incubated under conditions described under *Materials and Methods* except that incubation times were varied as shown. ●, No addition; ▲, 50 $\mu\text{g/ml}$ NIH-FSH-S12. Note the scale differences between panels A and B.

Effect of development on responsiveness of adenylyl cyclase to fluoride ion

Since FSH-mediated responsiveness of adenylyl cyclase activity in homogenates of SCE testes diminished as a function of age, the sensitivity of the cyclase system to fluoride ion (nonreceptor mediated) was determined. In Fig. 5, cyclase sensitivity to fluoride was tested in homogenates of SCE testes from animals of varying ages. Fluoride (10 mM) increased adenylyl cyclase activity 9.5-fold in preparations from 5- to 10-day-old animals: from 17 pmol cAMP to 160 pmol cAMP formed/min·mg protein. Although basal and fluoride-stimulated cyclase activity decreased with increasing animal age, fluoride stimulated the enzymatic activity 4-fold in testes from SCE animals 25 days of age and older. Even at 45 days the response to NaF was significant at $P < 0.01$. It was concluded from these results that catalytic activity of

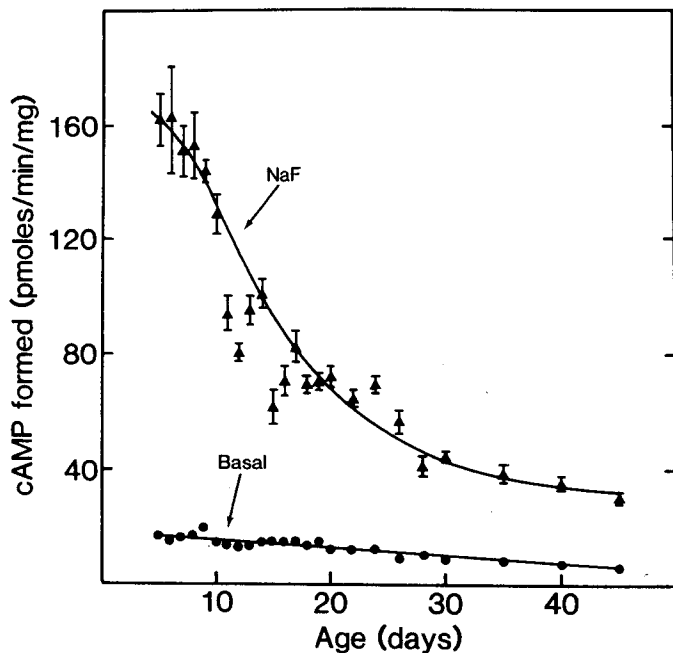


FIG. 5. Fluoride responsiveness of SCE testis homogenate adenylyl cyclase as a function of animal age. Adenylyl cyclase activity was determined in the absence (●) or presence (▲) of 10 mM NaF. Assay conditions were as described in *Materials and Methods*. Means \pm SE from three separate experiments are shown for the NaF data. The basal data are identical to those depicted in Fig. 1. Because of the change in scale, SE cannot be graphed.

adenylyl cyclase capable of stimulation existed throughout testis development.

Effect of fluoride on adenylyl cyclase in homogenates of immature and mature SCE testis

A separable regulatory subunit that is modulated by guanine nucleotides (GTP) and fluoride has been demonstrated to interact with the catalytic moiety of cyclase (22, 23, 27, 33). The decreased ability of fluoride to cause a large (10- to 15-fold) stimulation of adenylyl cyclase during later phases of testis maturation led us to determine whether a shift in the affinity of this regulatory protein for fluoride occurred with age. The dose dependence of fluoride effects on adenylyl cyclase in testicular homogenates from 10- and 45-day-old SCE animals is shown in Fig. 6. Fluoride activated the enzyme system in preparations from 10-day-old animals in a dose-dependent manner (*panel A*). Half-maximal activation of the enzyme was reached at 12.5 mM NaF and maximal stimulation occurred at 25 mM NaF. Activity was increased 8.5-fold from 10 pmol cAMP to 85 pmol cAMP formed/min·mg protein. At fluoride concentrations greater than 25 mM, activity was markedly inhibited. The dose dependence of fluoride activation of adenylyl cyclase in preparations from 45-day-old animals (*panel B*) was similar to that seen in testicular homogenates from im-

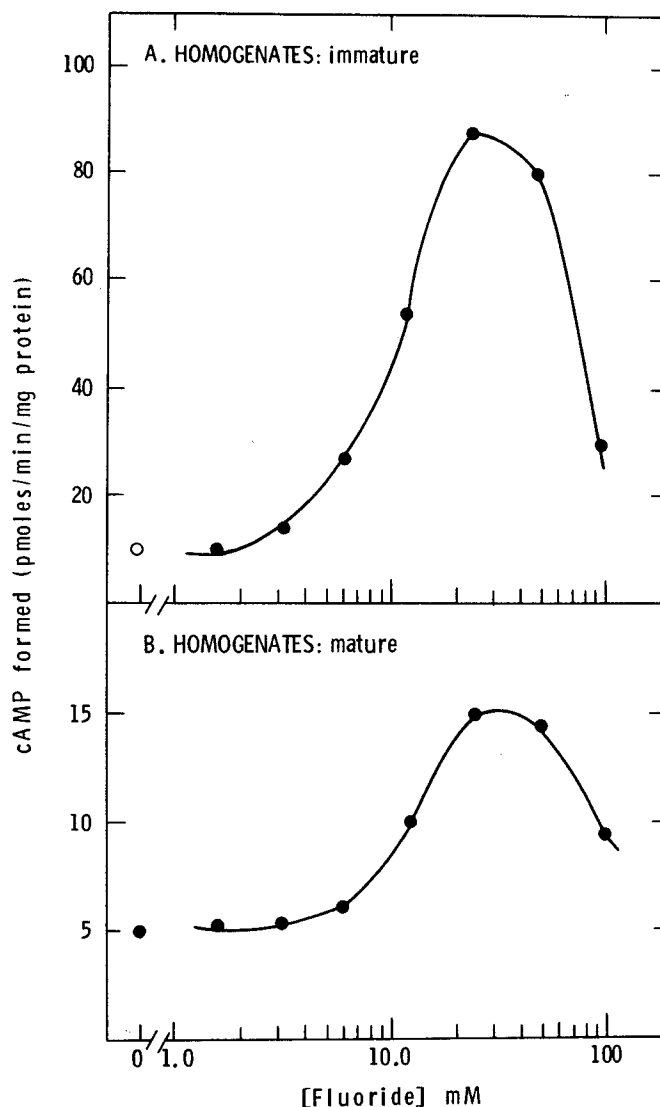


FIG. 6. Fluoride effect on adenylyl cyclase activity in homogenates of immature and mature SCE testes. Cyclase activity was determined in testicular homogenates of 12-day-old (A) and 45-day-old (B) SCE rats in the presence of varying concentrations of NaF. Again note the scale differences between the A and B panels. Assay conditions were as described in *Materials and Methods*.

mature animals. Maximal activation of the enzyme was also obtained at 25 mM fluoride, but the degree of stimulation was only 3-fold, from 4.5 pmol cAMP to 15 pmol cAMP formed/min·mg protein. These observations imply that the maximally effective concentration of fluoride for adenylyl cyclase activation is identical in preparations from immature and mature SCE animals, *i.e.* no shift in apparent affinity of the regulatory protein for fluoride occurs with increasing animal age.

Effect of guanine nucleotides on responsiveness of adenylyl cyclase to FSH

Because GTP binding proteins are components of the molecular complex that comprises the adenylyl cyclase

system and appear to facilitate hormone-cyclase interactions, we examined the effect of a nonmetabolizable GTP analog, GMP-P(NH)P, on basal and FSH-stimulated activity in testicular homogenates from 10- and 50-day-old SCE animals (Fig. 7). GMP-P(NH)P (10^{-4} M) enhanced basal activity in preparations from immature animals (*panel A*) 3.8-fold, increasing cAMP formed from 8 to 30 pmol/min·mg protein. NIH-FSH-S12 (50 μ g/ml) stimulated basal adenylyl cyclase activity 2.2-fold

in the absence of GMP-P(NH)P. In the presence of 10^{-4} M GMP-P(NH)P, an identical degree of FSH stimulation of basal activity was obtained, 2.3-fold over basal. In contrast, GMP-P(NH)P reestablished a hormonal response in testicular homogenates of mature animals (*panel B*). Adenylyl cyclase in these preparations is insensitive to FSH in the absence of GMP-P(NH)P. At 2×10^{-4} M GMP-P(NH)P, basal and FSH-stimulated activity were enhanced from 6.2 pmol cAMP to 12 and 27 pmol cAMP formed/min·mg protein, respectively. It appears from these results that a GTP analog nonhydrolyzable by terminal phosphatases restores the sensitivity of testicular adenylyl cyclase to FSH in mature animals while not affecting the relative hormonal responsiveness in homogenates from immature animals.

Effect of FSH on adenylyl cyclase activity in testicular membrane preparations from immature and mature animals

In an attempt to investigate the effect of GMP-P(NH)P on hormonal responsiveness of adenylyl cyclase in a more defined manner, washed membrane particles were prepared from SCE testes. Membrane fractions were prepared as described in *Materials and Methods* with the assumption that endogenous, loosely bound guanine nucleotides would be removed. In contrast to homogenates, FSH stimulated adenylyl cyclase in membrane preparations from testes of mature SCE rats. The dose dependence of FSH activation of adenylyl cyclase was tested in testicular membranes prepared from 12- and 55-day-old SCE animals (Fig. 8). In preparations from immature animals (*panel A*), 50 μ g/ml NIH-FSH-S12 stimulated activity maximally from 5.5 pmol cAMP to 13.0 pmol cAMP formed/min·mg protein (2.5-fold stimulation) and in membrane preparations from testes of mature animals activity also increased in response to FSH. A maximal increase from 3.3 to 9.5 pmol cAMP formed/min·mg protein was observed at 100 μ g FSH/ml.

Time course of adenylyl cyclase activity in testicular membrane preparations from immature and mature SCE animals

Adenylyl cyclase activity was assayed in membrane preparations of testes from 12- and 45-day-old SCE animals (Fig. 9). In preparations from immature animals, enzyme activity was linear for 18 min in the absence or presence of 50 μ g/ml NIH-FSH-S12 (*panel A*), with FSH eliciting a 2.3-fold stimulation of adenylyl cyclase activity. In contrast to the lack of demonstrable hormone-sensitive activity in testicular homogenates of mature animals, a 3.5-fold stimulation of activity by FSH occurred in washed membrane preparations from testes of mature animals (*panel B*). The inability of adenylyl

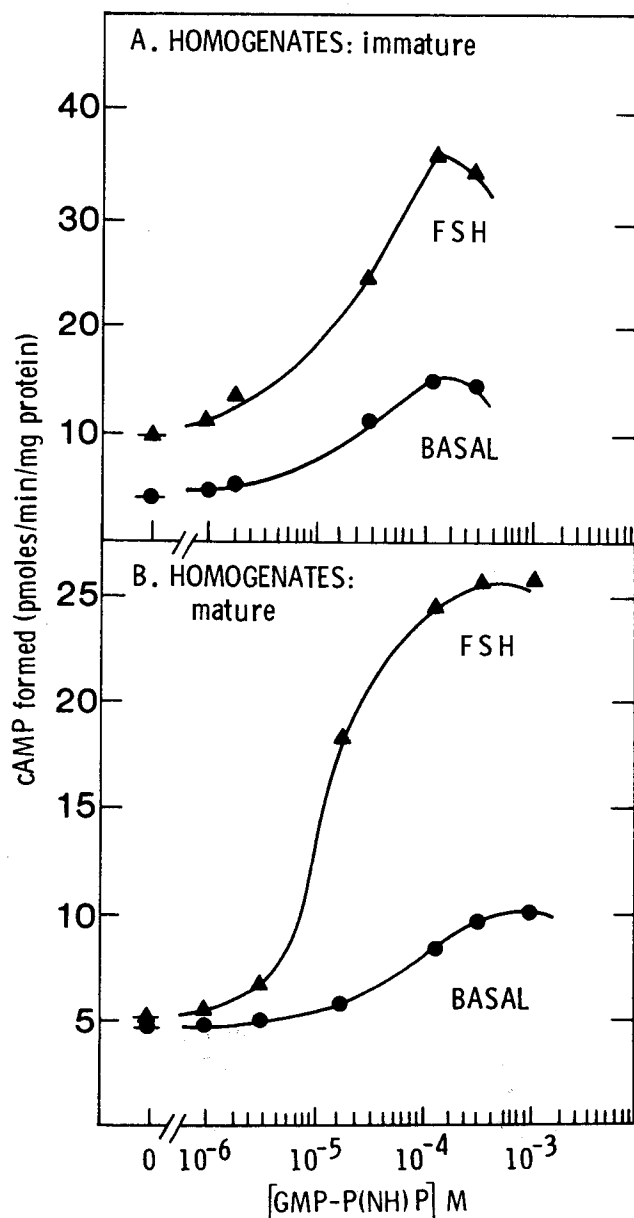


FIG. 7. Effect of GMP-P(NH)P on SCE testis adenylyl cyclase. Adenylyl cyclase activity was determined in testicular homogenates prepared from SCE rats 10 days (A) and 45 days (B) of age. Enzyme activity in the absence (●) or presence (▲) of 50 μ g/ml NIH-FSH-S12 was assayed with varying concentrations of GMP-P(NH)P. Assay conditions were as described in *Materials and Methods*. The scales used in panels A and B are different.

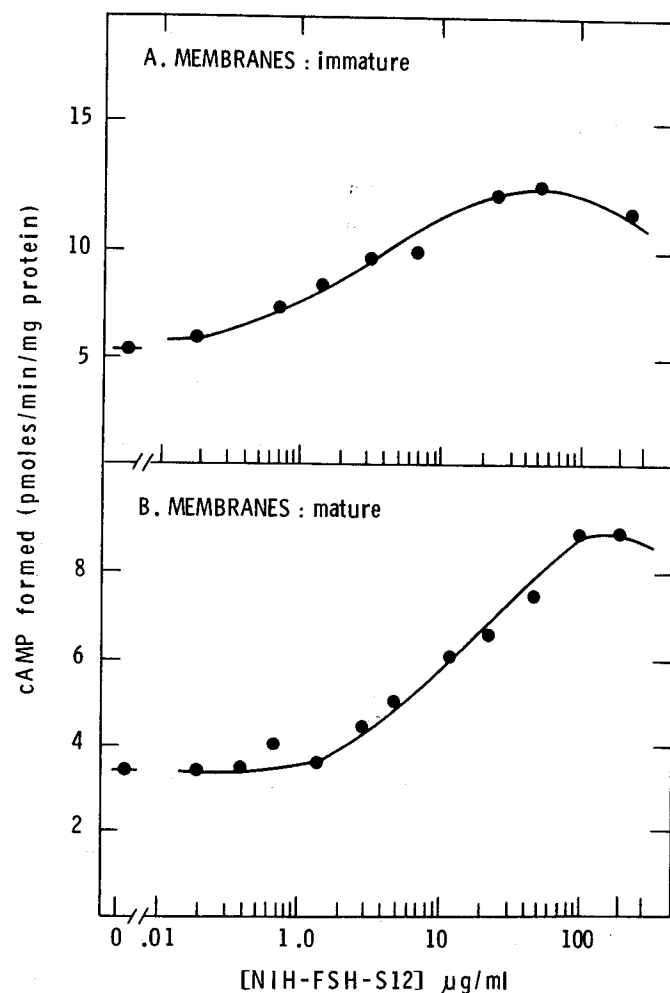


FIG. 8. Effect of FSH on adenylyl cyclase activity in testicular membrane preparations. Adenylyl cyclase activity was determined in membranes prepared from 12-day-old (A) and 55-day-old (B) SCE rat testes as described in *Materials and Methods*. Enzyme activity was assayed in the presence of varying concentrations of NIH-FSH-S12 under assay conditions described in *Materials and Methods*.

cyclase to respond to FSH in testicular homogenates from mature animals suggests that factors removed during membrane preparations may be potentially responsible for the decrease in hormone sensitivity as a function of testes maturation.

Effect of guanyl nucleotides on hormonal responsiveness of adenylyl cyclase in membrane preparations of testis from immature and mature animals

Because the GTP analog GMP-P(NH)P restores adenylyl cyclase responsiveness to FSH in testicular homogenates from mature animals, we tested the effect of this agent on hormone responsiveness of cyclase in membrane preparations from immature and mature SCE testes (Fig. 10). Adenylyl cyclase activity in testicular membrane preparations from 12-day-old animals was

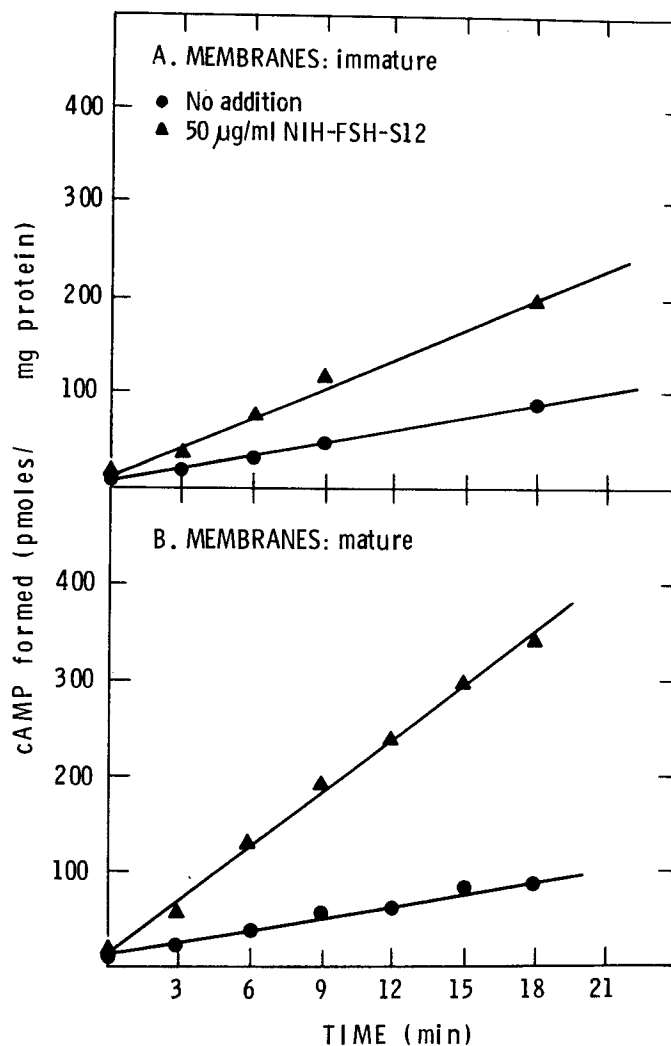


FIG. 9. Time course of adenylyl cyclase activity in membrane preparations of SCE testis. Activity was assayed in testicular membrane preparations of 12-day-old (A) and 45-day-old (B) old SCE rats as described in *Materials and Methods* in the absence (●) or presence (▲) of 50 µg/ml NIH-FSH-S12. Incubation times were varied from 0–18 min.

stimulated 1.7-fold by FSH in the absence of GMP-P(NH)P (*panel A*). GMP-P(NH)P enhanced basal and FSH-stimulated activity in a dose-dependent manner to the same relative degree. In the presence of 5×10^{-6} M GMP-P(NH)P, activity was stimulated 1.4-fold by FSH. In similar preparations from mature animals, 10^{-6} M GMP-P(NH)P enhanced basal and FSH-stimulated adenylyl cyclase activity maximally (*panel B*). In the absence of nucleotide, FSH elicited a 2.3-fold stimulation of enzyme activity, and in the presence of 10^{-6} M GMP-P(NH)P, FSH stimulated adenylyl cyclase activity 2.7-fold over basal. In contrast to findings with testicular homogenates of mature testes, hormone responsiveness is not increased by addition of GMP-P(NH)P to membrane preparations of either immature or mature testes.

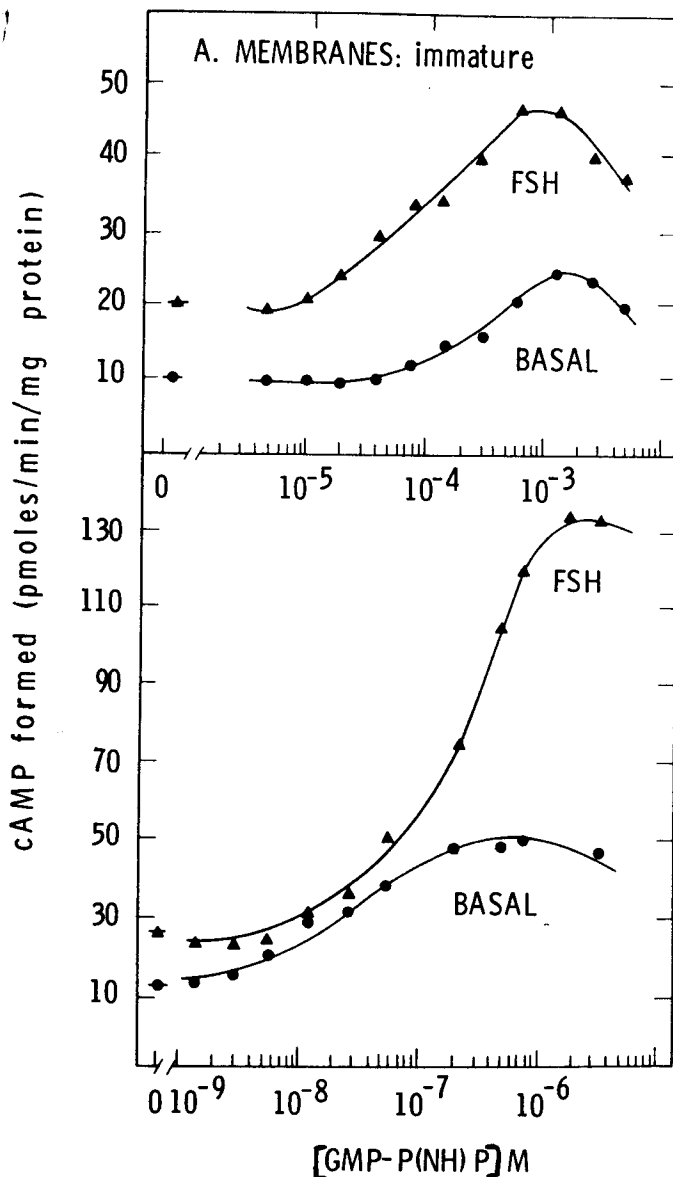


FIG. 10. Effect of GMP-P(NH)P on adenylyl cyclase in testicular membrane preparations. Activity in membrane preparations from testes of 12-day-old (A) and 50-day-old (B) SCE rats was determined in the absence (●) or presence (▲) of 50 $\mu\text{g/ml}$ NIH-FSH-S12 and varying concentrations of GMP-P(NH)P. Details of incubations are described in *Materials and Methods*.

The time course of GMP-P(NH)P activation of basal- and hormone-stimulated adenylyl cyclase was also determined in testicular membrane preparations from immature and mature SCE animals. Characteristic lag periods in basal enzyme activation by 50 μM GMP-P(NH)P were observed to be 3 and 9 min in membrane preparations from immature and mature testes, respectively. In the presence of 50 $\mu\text{g/ml}$ NIH-FSH-S12, the rate of GMP-P(NH)P activation was enhanced in both preparations. The apparent V_{max} of the enzyme was increased by GMP-P(NH)P both in the absence and in the presence of hormone (not shown).

Discussion

Results presented here suggest that the age-dependent loss of Sertoli cell responsiveness to FSH may involve either the capacity of receptor to activate the adenylyl cyclase, or of adenylyl cyclase to respond to the FSH-occupied receptor. Under the assay conditions used, adenylyl cyclase activity was stimulated 2- to 3-fold by FSH in crude testicular homogenates from immature SCE rats, whereas the enzyme in similar preparations from mature animals was unresponsive to FSH. The lack of FSH response in homogenates of mature SCE testes was not due to the presence of FSH-degrading enzymes, since 1) no effect could be seen even at concentrations as high as 500 $\mu\text{g/ml}$ of added NIH-FSH, and 2) at saturating concentrations of FSH the effect was absent from the outset of the incubation. The observed disappearance of sensitivity of the homogenate adenylyl cyclase to FSH as a function of testis maturation correlates with the investigations of Steinberger *et al.* (20). Accumulation of cAMP in isolated Sertoli cells from mature rats in the presence of FSH and the phosphodiesterase inhibitor methylisobutylxanthine was only 10% of the levels of cAMP accumulated by cells from immature animals in the presence of FSH alone. The possibility of the presence of an isoform of phosphodiesterase in the mature rat testis that is resistant to methylisobutylxanthine has been ruled out (7, 34). The lack of FSH stimulation of adenylyl cyclase in testicular homogenates from mature rats is not due to alteration in the catalytic activity of the system, which remains demonstrable throughout development. Fluoride maintained a 4-fold stimulation of the enzyme presumably via a nonspecific (nonreceptor mediated) mechanism in homogenates from testes of 45-day-old SCE rats. It is also interesting to note that the decrease in basal adenylyl cyclase activity with advancing age observed in testes homogenates is not noted in membrane preparations. This is readily seen from the scale differences in Figs. 4, 6, and 7, where homogenates were used, compared with the similar scales of Figs. 8-10, where membrane particles were the source of adenylyl cyclase activity. The decrease in homogenate basal activity correlates in a positive manner with the data of Steinberger *et al.* (20) concerning cAMP accumulation.

The involvement of age-dependent changes in receptor-cyclase coupling in homogenates from immature and mature testes was explored indirectly by determining effects of GMP-P(NH)P on basal and FSH-stimulated adenylyl cyclase activity. GMP-P(NH)P has been shown to enhance basal and hormone-stimulated cyclase activity in a wide variety of animal tissues (35). GMP-P(NH)P had no effect on the degree of responsiveness of adenylyl cyclase to FSH in homogenates from testes of immature animals, but promoted the appearance of a response to

FSH in homogenates from mature animals. Thus, FSH-stimulated activity in testicular homogenates of 45-day-old animals was increased 400% in the presence of 3×10^{-5} M GMP-P(NH)P in contrast to a 200% increase in basal activity. Of interest was the finding that FSH elicited a stimulation of adenylyl cyclase activity in both the mature and the immature systems, provided that it was tested in membrane particles as opposed to crude homogenates.

The mechanism of the age-related loss of FSH responsiveness and the uncovering of hormone stimulation either by preparation of membrane particles or by addition of GMP-P(NH)P to testicular homogenates is not known and a matter for speculation. The role of nucleotides in receptor coupling to adenylyl cyclases is complex (36-49). Coupling is stimulated by GTP (37, 39-41, 44-47), often inhibited by guanosine diphosphate (45-47). GTPase has been implicated at the GTP-binding regulatory site of the system (47-49). It is possible that the age-related loss of receptor-cyclase coupling is due to a stimulation of the putative GTPase activity with concomitant inhibition of hormonal stimulation. In agreement with this possibility, GMP-P(NH)-P, which is not susceptible to the GTPase activity, overcame the age-related regulation of the system. On the other hand, it has been shown kinetically (44) that receptor signal transduction is more efficacious when the nucleotide present is GMP-P(NH)-P than when it is GTP or guanosine diphosphate (44, 46). This increased effectiveness in hormonal stimulation can be seen with respect to degree of stimulation (e.g. percent of possible V_{max} under the assay conditions used) and sometimes also with respect to apparent hormone potency (46). Since FSH stimulation was observed in homogenates of mature SCE rat testes upon GMP-P(NH)P addition, it is clear that the receptor *per se* is not inactive. A factor present in cytosol capable of regulating receptor activity and/or GTPase activity could explain the results obtained.

In view of the fact that incubation conditions were such as to allow for maintenance of at least 85% of added GTP (see *Materials and Methods*), it would appear that the decreased FSH stimulation seen in homogenates of mature SCE testes is not due to excessive hydrolysis of "bulk" regulatory GTP. The finding that the lack of FSH responsiveness did not "develop" as a function of incubation time is also consistent with the notion that the lack of response is due to presence of an interfering factor (or factors) acting at the level of either the adenylyl cyclase-related GTPase or the receptor signal generation, but not acting by nonspecifically hydrolyzing regulatory GTP.

It was noted that relative stimulation of activity by FSH appeared to be greater in membranes obtained from "nonresponsive" homogenates of mature SCE testes than

in membranes from the "responding" homogenates of immature SCE testes. An explanation for this rather paradoxical finding is not apparent to us. This "inversion" in responsiveness upon washing was noted in all our experiments, even though absolute cyclizing activities tended to vary by as much as 3-fold from experiment to experiment.

In our experiments GMP-P(NH)P had no synergistic effect on FSH-stimulated activity in membrane preparations from either mature or immature animals, rather it increased basal- and hormone-stimulated activity to the same relative degree. These results are in agreement with the observation of Maghoun-Rogister and Hennen (50) that GMP-P(NH)P did not potentiate the degree of responsiveness to FSH of adenylyl cyclase in porcine testicular membranes from immature animals. In contrast, Reichert and Abou-Issa (51) have reported that GMP-P(NH)P augments FSH effects on adenylyl cyclase in membranes from testis of immature rats, indicating better coupling in their system. This difference is probably due to the use of different membrane preparations.

In conclusion, the data presented here indicate that concomitant with testicular maturation, the Sertoli cell FSH-sensitive adenylyl cyclase system seen in testicular homogenates loses its FSH responsiveness. This loss of responsiveness can be "cured" *in vitro* in two ways: 1) addition of the potent GTP analog GMP-P(NH)P; and 2) removal of soluble component(s) by preparation of membrane particles. In view of the fact that SCE testes are composed not only of Sertoli cells, but contain also up to 8% of other cell types including Leydig and myoid cells, the cellular origin of the interfering factor cannot be established at this time. Both the origin and the nature of this factor are currently under investigation.

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